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(54) Title: METHODS FOR NEURAL DIFFERENTIATION OF EMBRYONIC STEM CELLS USING PROTEASE PASSAGING TECHNIQUES

(57) Abstract: The present invention provides methods for human pluripotent cell culturing and for neural cell production. More particularly, the present invention provides culturing methods employing dissociating cell cultures to an essentially single cell culture, such as by employing antibody selection and bulk passaging treatments utilizing the subsequent application of Collagenase and trypsin. In certain embodiments, the cells are further treated with essentially serum free MEDII conditioned medium, proline, or minimal medium, and are optionally treated with amphiphilic lipid compounds for the generation of human neural cells from pluripotent human cells. In certain embodiments, the cells cultured using these methods have an abnormal karyotype.



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# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/10121

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 5/00, 15/85, 15/86; A01N 1/02

US CL : 435/325, 1.1, 405

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/325, 1.1, 405

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
60/459,090

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
EAST, PALM

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2002/016794 (Wernet) 07 November 2002 (07.11.2002), entire reference.	1-41, 44-85 ----- 42-43, 86, 87
X --- Y	US 2002/0160510 (HARIRI) 31 October 2002 (31.10.2002), entire reference.	1-41, 44-85 ----- 42, 43, 86, 87
X --- Y	US 6,200,806 (THOMSON) 13 March 2001 (13.03.2001), entire reference.	1-41, 44-85 ----- 42, 43, 86, 87
X --- Y	US 5,843,780 (THOMSON) 01 December 1998 (01.12.1998), entire reference.	1-41, 44-85 ----- 42, 43, 86, 87



Further documents are listed in the continuation of Box C.



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"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

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**INTERNATIONAL SEARCH REPORT**International application No.  
PCT/US04/10121**C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y	US 6,280,718 (KAUFMANN et al.) entire reference.	1-41, 44-85 ————— 42, 43, 86, 86
A	US 2003,0166272 A1 (ABULJADAYEL) 04 September 2003 (04.09.2003), entire reference.	1-87

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## C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y	US 6,280,718 (KAUFMANN et al.) entire reference.	1-41, 44-85 ----- 42, 43, 86, 86
A	US 2003,0166272 A1 (ABULJADAYEL) 04 September 2003 (04.09.2003), entire reference.	1-87